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TECHNICAL MANUSCRIPT 316

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Louis E. Schneider

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DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

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Louis E. Schneider

Process Development Division
AGENT DEVELOPMENT AND ENGINEERING LABORATORY

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A SIMPLE, ALL-GLASS DEVICE FOR ISOLATING CLONE MAMMALIAN CELLS AND CLONE VIRUS

ABSTRACT

A simple and inexpensive all-glass device is described for use in the isolation of clone mammalian cells and clone virus.

Glass or plastic petri dishes are widely used for cloning mammalian cells. The method commonly involves plating an appreciable number of cells per dish, followed by the use of silicone-treated cylinders to isolate and retrieve selected clones. The technique is simple enough to perform, but the many manipulations involved in maintenance and retrieval of the clones are time-consuming and conducive not only to extraneous contamination, but also to internal cross-contamination of the clones.

These considerations led to the development of what is in effect a multi-chambered petri dish (Fig. 1). The bottom of a standard-sized (100 mm by 15 mm) glass petri dish is used as the floor of the device. Within this dish are placed two glass cylinders, each 45 mm high; the outer one is 85 mm in outside diameter (O.D.) and the inner is 45 mm O.D. These cylinders are prepared on a rotary glass-cutting saw from standard-sized pyrex tubing; the edges need not be fire-polished. Eighteen shell vials (1 dram size, 15 mm O.D., 45 mm high; for example, Kimble item 60930L, Owens-Illinois Glass Company, Toledo, Ohio) are placed between the walls formed by the cylinders: 13 in the outer ring, 5 in the inner one. The inner cylinder keeps the vials in two orderly circles. A second standard-sized glass petri dish bottom serves as the cap for the device.

Since the device is constructed entirely of glass, sterilization is simple with either wet or dry heat. The all-glass feature further permits complete visualization on inverted microscopes, and the compact size renders manipulation easy on even the smallest microscope stage. The low cost and simplicity of the device permit ready assembly and use of many units; the low cost of the shell vials permits single use. These features present distinct advantages over a metal rack* designed to hold

* Richter, A.; Halle, S. 1965. New rack for use in cell culture. Appl. Microbiol. 13:503-504. Also, Technical Manuscript 195. February 1965. Virus and Rickettsia Division, U.S. Army Biological Laboratories, Frederick, Maryland.

two rows of tape-sealed glass vials for viewing on an inverted microscope. However, the all-glass device as described is not gas-tight, so successful cloning requires either the use of a carbon dioxide incubator or the incorporation of tris(hydroxymethyl)aminomethane buffer in the medium.

In use, the vials are set with 1 or 2 ml of medium theoretically containing one mammalian cell. According to the Poisson distribution of a single particle per unit volume, 37% of the vials would have no cells, 37% only one cell, and 26% more than one cell. The cells are allowed to propagate for 2 weeks without change of medium, at which time the clones are quite obvious and vials with more than one clone are easily eliminated. The selected single clones can then conveniently be transferred to T-15 or T-30 flasks as the next stage in line build-up.

An anticipated further application is the isolation of clone virus. Monolayers of a cell line would be prepared in the vials, then infected with virus that had been diluted to one plaque-forming unit per unit volume of medium. Overlayering of the infected cells with an agar-nutrient medium mixture would enable the plaques to develop; staining the cells with a neutral red solution would permit ready selection of those vials containing only one plaque. Since the virus particle responsible for a single plaque in a vial would have been isolated from the start, the assurance of obtaining clone virus successfully with this method would be appreciably greater than with any other tissue culture method currently in use.

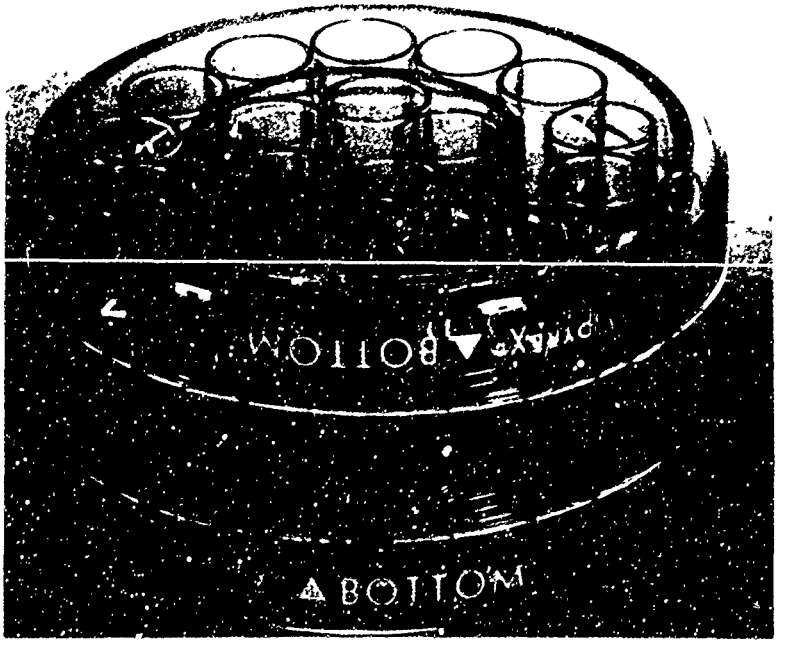

Figure 1. All-Glass Device for Isolating Clone Mammalian Cells and Clone Virus.

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